Growth of *Clostridium perfringens* from spore inocula in *sous-vide* turkey products[1]

Vijay K. Juneja*, Benne S. Marmer

Microbial Food Safety Research Unit, U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA

Received 13 September 1995; revised 29 December 1995

Abstract

Clostridium perfringens growth from a spore inoculum was investigated in vacuum-packaged, cook-in-bag ground turkey (pH 6) that included 0.3% (w/w) sodium pyrophosphate, and sodium chloride at 0, 1, 2 or 3% (w/w). The packages were processed to an internal temperature of 71.1°C, ice chilled and stored at various temperatures. The total *C. perfringens* population was determined by plating diluted samples on tryptose-sulfite-cycloserine agar followed by anaerobic incubation at 37°C for 48 h. At 28°C, the addition of 3% salt in turkey was effective in delaying growth for 12 h. At 15°C, growth occurred at a relatively slow rate in the presence of 1–2% salt. Vegetative cells were not observed even after 28 days of storage in the presence of 3% salt. *C. perfringens* growth was not observed at 4°C regardless of salt levels. The D-values ranged from 23.2 min (no salt) to 17.7 min (3% salt). Cyclic and static temperature abuse of refrigerated products for 8 h did not lead to growth by *C. perfringens* from a spore inoculum.

1. Introduction

Increasing consumer demands for minimally processed, ready-to-eat, extended shelf-life, refrigerated foods has led to growth in the use of *sous vide* (under vacuum) food processing technology. It is an advanced method of cooking whereby fresh food is vacuum sealed in heat-stable, high barrier plastic pouches or films, cooked (pasteurized) to a time and temperature for a specific food, chilled rapidly to avoid germination and outgrowth of surviving bacterial spores, and stored at refrigeration temperatures. However, a large proportion of the *sous-vide* products sold in North America are actually stored frozen.

According to Shamsuzzaman et al. (1992), commercial application of *sous vide* technology in the United States is limited due to microbiological food safety concerns and requirements for long shelf-lives, particularly if such products are subject to possible temperature abuse at some stage during transportation, distribution, storage or handling in supermarkets or by consumers. Sufficient evidence exists to document that temperature abuse is a common occurrence at both the retail and consumer levels. Wyatt and Guy (1980) reported that temperatures of supermarkets were consistently greater than 7.5°C, with some as high as 17.5°C, while temperatures of home refrigerators ranged from 1.7–20.2°C. Some *C. perfringens* strains can grow at temperatures as low as 6°C (Johnson, 1990). Therefore, while the safety and preservation of *sous vide* foods relies mainly on a mild heat treatment and chilled storage, secondary microbial barriers must be incorporated for safe marketing of *sous vide* products (RFMCC-NFPA, 1988; Notermans et al., 1990; Mossell and Struijk, 1991).

While Americans consume about ten billion pounds of processed meats each year (Marsden, 1980) and most of these products contain 1.1–1.3% sodium (2.75–3.25% NaCl) (Maurer, 1983), the topic of sodium in the American diet has become a major health issue. The primary concern is the association of the sodium electrolyte with hypertension or high blood pressure (Tobian, 1979; Kerr and Nichman, 1986). Sodium reduction has been recommended as an important step in the management of hypertension and symptoms associated with coronary heart disease and renal failure. Accordingly, the present work was initiated to examine (a) the growth potential of *C. perfringens* from a spore inoculum in *sous vide* turkey products at refrigerated and cyclic and static temperature abuse storage conditions, and (b) the heat resistance of *C. perfringens* spores in turkey containing 0–3% salt (sodium chloride) levels.

2. Materials and methods

2.1. Test organisms and spore production

Clostridium perfringens strains NCTC 8238, NCTC 8239 and ATCC 10288 were used in the study. The origin and sources of the strains and spore production methods have been reported (Juneja et al., 1993). After the spore crop of each

strain had been washed twice and resuspended in sterile distilled water, the spore suspensions were stored at 4°C. Spores of individual strains at equal numbers were then pooled to prepare a cocktail. This composite of spore strains was heat-shocked for 20 min at 75°C prior to use.

2.2. Source, preparation and inoculation of meat

Ground turkey was obtained from a local retail market and frozen (− 5°C) until use (approximately 40 days). Sodium pyrophosphate and salt was mixed into all ground turkey samples with a Hobart mixer to give a final concentration of 0.3% and 0, 1, 2, or 3%, (w/w), respectively. The pH of the ground turkey was determined using a combination electrode (Sensorex, semi-micro, A.H. Thomas, Philadelphia, PA) attached to an Orion model 601A pH meter and was adjusted to pH 6 with lactic acid. Duplicate 25-g ground turkey samples were aseptically weighed into filter stomacher bags (SFB-0410; Spiral Biotech., Bethesda, MD) and inoculated with 1 ml of the heat-shocked *C. perfringens* spore cocktail so that the final concentration of spores was approximately 2.5 $\log_{10}$ CFU/g. Thereafter, the bags were manually mixed to ensure an even distribution of the organisms in the meat sample. Negative controls consisted of bags containing uninoculated turkey. The bags were placed in 7 inch × 8 inch plastic barrier bags (Koch Model 01 46 09, Kansas City, MO). The oxygen transmission rate of the nylon/polyethylene film was 3.5 $cm^3/100\ inch^2$ in 24 h measured by the manufacturer at 75°F and 75% relative humidity. The bags were evacuated to a negative pressure of 1000 millibars and heat sealed using a Multivac Model A300/16 gas packaging machine (Germany).

2.3. Cooking and cooling protocols

The ground turkey samples were processed in 80°C water in a water circulating bath (Exacal, Model Ex-251HT, NESLAB Instruments, Inc., Newington, NH) to an internal temperature (monitored by thermocouples) of 71.1°C within 10 min and quickly cooled in an ice slurry as described previously (Juneja and Majka, 1995).

2.4. Storage, temperature abuse, and sampling

The inoculated samples were stored at 4, 15, and 28°C. Samples stored at 28°C were analyzed at 4, 8, 12, 16, 24, 48, and 72 h, those at 15°C were analyzed on day 1, 2, 3, 4, 6, and 7 and the samples stored at 4°C on day 7, 14, 21, and 28. To determine the effect of cyclic temperature abuse, samples stored at 4°C were transferred 7 days before plating on their scheduled sampling day (7, 14, 21, and 28 days) to 15°C, held at this temperature for 24 h, and then returned to 4°C and plated on their scheduled sampling day. To determine the effect of static temperature abuse, some samples stored at 4°C were moved 8, 12, and 20 h before plating on their scheduled sampling day (7, 14, 21, and 28 days) to 28°C.

2.5. Bacterial enumeration procedure

On the scheduled sampling day, samples were removed and enumerated for total *C. perfringens* population by spiral plating (Spiral Systems Model D plating instruments; Cincinnati, OH) on tryptose-sulfite-cycloserine (TSC) agar as described previously (Juneja et al., 1993). The total *C. perfringens* population was determined after 48 h of incubation at 37°C in a Gas Pak system (Baltimore Biological Laboratory, Cockeysville, MD). In addition, a 25 g portion of both uninoculated raw turkey and cooked turkey were used to verify the absence of naturally occurring *C. perfringens*. This involved the use of lactose-gelatin and nitrate-motility medium (Schwab et al., 1984).

2.6. Determination of spore heat resistance

A known weight of turkey was aseptically transferred to a sterile Waring blender and mixed with an equal volume of sterile distilled water by blending for 2 min to form a smooth paste. Turkey slurry containing 0.3% (w/w) sodium pyrophosphate and 0, 1, 2, or 3% (w/w) NaCl was inoculated with the heat-shocked *C. perfringens* spore cocktail to obtain an initial count of about 7 $\log_{10}$ spores/ml. Thermal inactivation was carried out at 99°C in sterile 17 × 60 mm screw-capped vials as described previously (Juneja and Majka, 1995). The surviving spore population was determined by spiral plating (Model D, Spiral Systems, Cincinnati, OH) on agar plates containing TSC supplemented with lysozyme (10 μg/ml; Sigma, 41 000 U/mg). The plates were incubated anaerobically at 28°C for 6 days to recover heat injured spores.

2.7. Data processing

Bacterial growth curves were generated from the experimental data using the Gompertz equation (Gibson et al., 1987) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-based program was developed by W.C. Damert (USA Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA). The Gompertz parameter values were subsequently used to calculate generation times and lag times as described by Gibson et al. (1987). The D-values (time for 10-fold reduction in viable spores) were estimated by computing the linear regression (Ostle and Mensing, 1975) of $\log_{10}$ number of survivors versus heating time using Lotus 1-2-3 Software (Lotus Development Corporation, Cambridge, MA) and taking the absolute value of the inverse slope.

3. Results and discussion

During storage at 28°C, *C. perfringens* spores germinated and grew from 2.25 to approximately 5 $\log_{10}$ CFU/g after 12 h in cooked ground turkey (pH 6) containing

no salt. (Fig. 1). In the presence of 1–2% salt, growth was relatively slow and the total *C. perfringens* population was < 4 $\log_{10}$ CFU/g after 12 h of storage at 28°C. Increasing the salt level to 3% completely arrested *C. perfringens* germination and growth for 12 h. However, the levels increased to > 5 $\log_{10}$ CFU/g after 16 h in all turkey samples regardless of the presence or absence of salt (Fig. 1). At 28°C, the

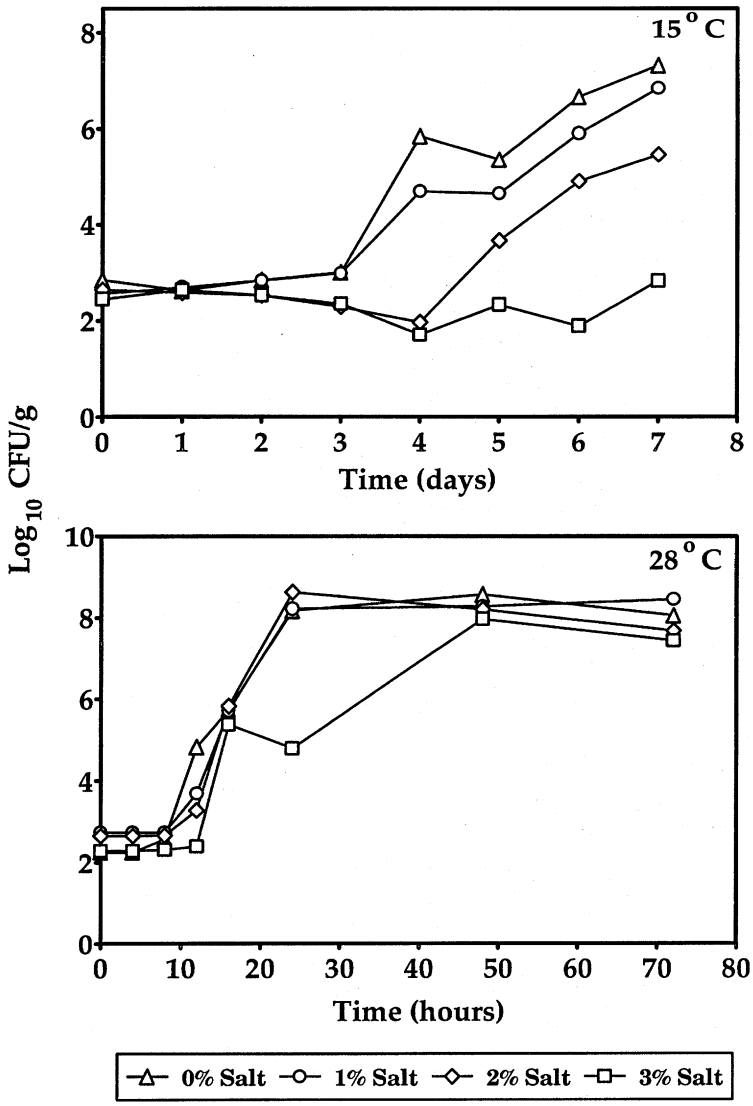

Table 1
Mean generation times and lag times[a] of *Clostridium perfringens* in ground turkey which contained 0.3% sodium pyrophosphate at pH 6 and salt levels of 0, 1, 2, and 3% salt

Salt (%)	Generation times (min)[b]		Lag times (h)	
	28°C	15°C	28°C	15°C
0	39.4	300.0	7.3	61.6
1	31.3	398.8	10.6	59.6
2	24.2	238.2	11.6	106.4
3	88.5	nd[c]	8.0	nd[c]

[a]Mean of two replications.
[b]Generation times calculated from regression lines for exponential growth using the Gompertz equation.
[c]Not determined.

generation times ranged from 39.4 min in salt-free turkey samples to 88.5 min in samples with 3% salt (Table 1). The lag times were 7.3 and 8.0 h, respectively. In a study by Juneja and Majka (1995), the generation and lag times of *C. perfringens* spores at 28°C ranged from 80.1 min to 11.55 h in cook-in-bag ground beef (pH 7) with no salt; the values were 129.2 min and 27.53 h in samples at pH 5.5 with 3% salt. Pivnick et al. (1968) cooked chicken, containing 4 $\log_{10}$ spores/g, for barbecuing to an internal temperature of 85–90°C and stored it at 45°C. They reported a lag period of 4 h followed by logarithmic growth which proceeded at the same rate as that observed for vegetative cells in inoculated samples.

By day 4 at 15°C, *C. perfringens* spores germinated and grew to $> 5 \log_{10}$ CFU/g in turkey with no salt (Fig. 1). While the presence of 3% salt in samples at 15°C completely inhibited the germination and subsequent multiplication of vegetative cells even after 7 days of storage, growth occurred at a relatively slow rate in the presence of 1–2% salt. However, the total *C. perfringens* population was consistently lower as compared to the levels in turkey with no salt (Fig. 1). In contrast to 28°C, *C. perfringens* exhibited a 7.5 times longer generation time (300.0 min) and 8 times longer lag time (61.6 h) at 15°C in samples with no salt (Table 1). Juneja and Majka (1995) studied the fate of the *C. perfringens* spores in cook-in-bag ground beef at 15°C and reported generation and lag times of 415.9 min and 96.06 h, respectively.

During storage at 4°C, *C. perfringens* growth from a spore inoculum was not observed in turkey samples regardless of the presence or absence of salt (data not shown). Similar observations were reported by Juneja and Majka (1995) who cooked *C. perfringens* inoculated beef in a water bath to an internal temperature of 71.1°C and stored it at 4°C.

To determine the effect of static temperature abuse of refrigerated turkey products which may occur during storage, distribution, display or consumer handling of refrigerated food product, turkey samples stored at 4°C were moved to a 28°C environment for 8, 12, or 20 h before plating on their scheduled sampling day (7, 14, 21, and 28 days). *C. perfringens* population in samples abused for 8 h

did not increase regardless of the presence or absence of salt (Fig. 2). When samples were transferred 12 h before plating to 28°C, *C. perfringens* spores germinated, and grew to >6 $\log_{10}$ CFU/g only in samples with no salt (Fig. 2). There was no increase in the numbers of organisms in samples that contained 3% salt. However,

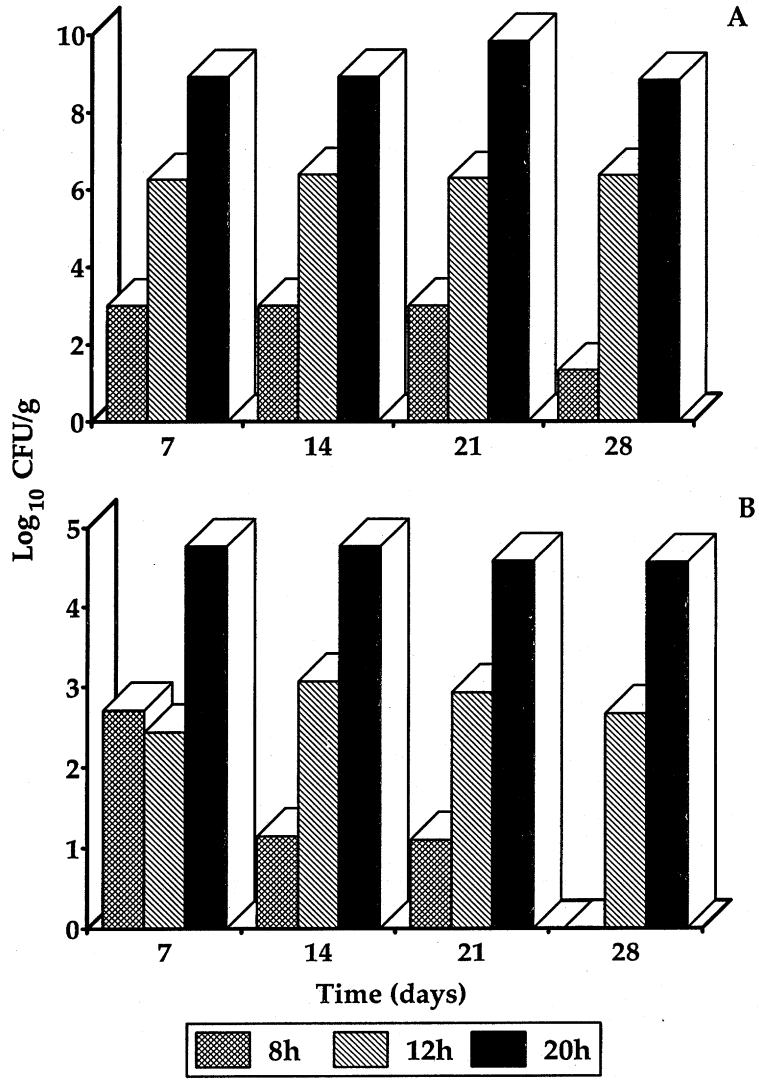

Fig. 2. The effect of static temperature abuse on growth of *Clostridium perfringens* from a spore inoculum in vacuum-packaged, cook-in-bag ground turkey (pH 6), that included 0.3% sodium pyrophosphate and 0 or 3% salt. The refrigerated samples were moved to 28°C for 8, 12 or 20 h before plating on their scheduled sampling day (7, 14, 21, and 28 days). (A) No salt; (B) 3% salt.

Table 2
Mean[a] *D*-values ± standard deviation at 99°C of spore cocktail of *Clostridium perfringens* strains NCTC 8238, NCTC 8239 and NCTC 10288 suspended in turkey slurry that included 0.3% sodium pyrophosphate at pH 6 with 0, 1, 2, or 3% salt

Salt	*D*-value at 99°C (min)
0	23.2 ± 0.2
1	21.3 ± 0.8
2	19.5 ± 0.8
3	17.7 ± 0.3

[a]Mean of two replications.

20 h abuse of turkey samples with 3% salt resulted in an increase in cell numbers of approximately 2 $\log_{10}$ CFU/g; the levels in all samples were < 5 $\log_{10}$ CFU/g (Fig. 2). To determine the effect of cyclic temperature abuse, samples stored at 4°C were transferred to 15°C for 24 h, 7 days before their scheduled sampling day (7, 14, 21, and 28). These samples showed no growth regardless of the presence or absence of salt (data not shown).

The thermal resistance of *C. perfringens* spores (expressed as *D*-values in minutes) in turkey slurries that included 0.3% sodium pyrophosphate at pH 6.0, and salt levels of 0, 1, 2, or 3% are shown in Table 2. The *D*-values at 99°C decreased from 23.32 (no salt) to 17.7 min (3% salt) at 99°C. In beef slurry, the values ranged from 23.33 (pH 7.0, 3% salt) to 14.00 min (pH 5.5, 3% salt) at 99°C (Juneja and Majka, 1995). In a study by Bradshaw et al. (1977), *D*-values at 99°C for *C. perfringens* spores suspended in commercial beef gravy ranged from 26 to 31.4 min. Differences in *D*-values obtained in our study and those reported by previous workers may be attributed to several factors. Sporulation media and the temperature used for spore preparation, heating menstruum, recovery conditions including the composition and pH of the medium, the presence of inhibitors, temperature and time of incubation, and above all, the presence or absence of lysozyme in the recovery media, affect the calculated spore heat resistance (Foegeding and Busta, 1981; Russell, 1982). Scott and Bernard (1982) suggested that there may be significant variations among strains, and the reported *D*-values by different investigators within the same strain.

While addition of increasing levels (1–3%) of salt in turkey can result in a parallel increase in sensitivity of *C. perfringens* spores at 99°C, it is practically not feasible to inactivate the spores by heat. A thermal process, if designed to inactivate *C. perfringens* spores, may impact the product quality negatively. Spores are likely to survive the normal pasteurization/cooking temperatures applied to *sous vide* foods. For growth of *C. perfringens* to occur in *sous vide* foods, four criteria must be met: activation, germination, outgrowth of spores, and vegetative growth. Mild heat treatment given to *sous vide* foods could serve as an activation step if it were not lethal to the spores. In a study by Barnes et al. (1963), about 3% of spores germinated in raw beef without prior heat shock, but almost all germinated after the meat was heated. Spores germinate at a reduced rate without prior heat shock (Craven, 1980).

In conclusion, our study has shown that *C. perfringens* may germinate and grow to unsafe levels if *sous vide* products are temperature abused for a relatively long period. For vacuum-packaged, cook-in-bag turkey products that contained 0.3% sodium pyrophosphate, cyclic and static temperature abuse for ≤ 8 h did not lead to *C. perfringens* growth from spore inocula. An extra degree of safety may be assured in such products by supplementation with 2–3% salt.

References

Barnes, E.M., Despaul, J.E. and Ingram, M. (1963) The behavior of a food-poisoning strain of *Clostridium welchii* in beef. J. Appl. Microbiol. 26, 415–427.

Bradshaw, J.G., Peeler, J.T. and Twedt, R.M. (1977) Thermal inactivation of ileal loop-reactive *Clostridium perfringens* type A strains in phosphate buffer and beef gravy. Appl. Environ. Microbiol. 34, 280–284.

Craven, S.E. (1980) Growth and sporulation of *Clostridium perfringens*. Food Technol. 34(4), 80–87.

Foegeding, P.M. and Busta, F.F. (1981) Bacterial spore injury: an update. J. Food Protect. 44, 776–786.

Gibson, A.M., Bratchell, N. and Roberts, T.A. (1987) The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62, 479–490.

Johnson, E.A. (1990) *Clostridium perfringens* food poisoning. In: Cliver D.O. (Ed.) Foodborne Diseases, pp. 229–240. Academic Press, San Diego, CA.

Juneja, V.K., Call, J.E. and Miller, A.J. (1993) Evaluation of methylxanthines and related compounds to enhance *Clostridium perfringens* sporulation using a modified Duncan and Strong medium. J. Rapid Methods Automation Microbiol. 2, 203–218.

Juneja, V.K. and Majka, W.M. (1995) Outgrowth of *Clostridium perfringens* in cook-in-bag beef products. J. Food Safety 15, 21–34.

Kerr, G.R. and Nichman, M.Z. (1986) Salt and hypertension. Public Health Rev. 14, 25–104.

Marsden, J.L. (1980) Sodium containing additives in processed meats. A technological overview. In: White P.L. and Crocco S.C. (Ed.) Sodium and Potassium in Foods and Drugs, pp. 49–59. Conference Sponsored by the Am. Med. Assoc. Chicago, IL.

Maurer, A.J. (1983) Reduced sodium usage in poultry muscle foods. Food Technol. 37, 60–64.

Mossell, D.A.A. and Struijk, C.B. (1991) Public health implication of refrigerated pasteurized (sous-vide) foods. Int. J. Food Microbiol. 13, 187–206.

Notermans, S., Dufrenne, J. and Lund, B.M. (1990) Botulism risk of refrigerated, processed foods of extended durability. J. Food Prot. 53, 1020–1024.

Ostle, B. and Mensing, R.W. (1975) Statistics in Research. Iowa State University Press, Ames, IA.

Pivnick, H., Erdman, I.E., Manzatiuk, S. and Pommier, E. (1968) Growth of food poisoning bacteria on barbecued chicken. J. Milk Food Technol. 31, 198–201.

RFMCC-NFPA, Refrigerated Foods and Microbiological Criteria Committee of the National Food Processors Association (1988) Factors to be considered in establishing good manufacturing practices for the production of refrigerated foods. Dairy Food Sanit. 8, 288–291.

Russell, A.D. (1982) The Destruction of Bacterial Spores. Academic Press, London.

Schwab, A.H., Leininger, H.V. and Powers, E.M. (1984) Media, reagents, and strains. In: Speck M.L. (Ed.) Compendium of Methods for the Microbiological Examination of Foods, 2nd edn., pp. 788–897. American Public Health Association, Washington, DC.

Scott, V.N. and Bernard, D.T. (1982) Heat resistance of spores of non-proteolytic type B *Clostridium botulinum*. J. Food Prot. 45, 909–912.

Shamsuzzaman, K., Chuaqui-Offermanns, N., Lucht, L., McDougall, T. and Borsa, J. (1992) Microbiological and other characteristics of chicken breast meat following electron-beam and *sous-vide* treatments. J. Food Prot. 55, 528–533.

Tobian, L. (1979) The relationship of salt to hypertension. Am. J. Clin. Nutr. 32, 2739–2748.

Wyatt, L.D. and Guy, V. (1980) Relationships of microbial quality of retail meat samples and sanitary conditions. J. Food Prot. 43, 385–389.